

**CORRECTED
VERSION***

PCT

WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau



INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

| | | |
|--|-----------|--|
| (51) International Patent Classification ⁶: G01N 15/10, 33/50, 15/14, C12Q 1/68 | A3 | (11) International Publication Number: WO 97/14028 (43) International Publication Date: 17 April 1997 (17.04.97) |
| (21) International Application Number: PCT/US96/16198 (22) International Filing Date: 10 October 1996 (10.10.96) (30) Priority Data: 08/540,814 11 October 1995 (11.10.95) US 08/542,401 11 October 1995 (11.10.95) US (71) Applicant (for all designated States except US): LUMINEX CORPORATION [US/US]; 12212 Technology Boulevard #K, Austin, TX 78727-6115 (US). (72) Inventors; and (75) Inventors/Applicants (for US only): CHANDLER, Van, S. [US/US]; 2808 McKinney Avenue #410, Dallas, TX 75204 (US). FULTON, R., Jerrold [US/US]; 305 Evergreen Trail, Cedar Hill, TX 75104 (US). CHANDLER, Mark, B. [US/US]; 4 Niles Road, Austin, TX 78700 (US). (74) Agent: PATTERSON, Melinda, L.; P.O. Box 4433, Houston, TX 77210 (US). | | (81) Designated States: AL, AM, AT, AU, BA, BB, BG, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, US, UZ, VN, ARIPO patent (KE, LS, MW, SD, SZ, UG), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG). Published <i>With international search report.</i> <i>Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i> (88) Date of publication of the international search report: 25 September 1997 (25.09.97) |
| (54) Title: MULTIPLEXED ANALYSIS OF CLINICAL SPECIMENS APPARATUS AND METHOD (57) Abstract A method for the multiplexed diagnostic and genetic analysis of enzymes, DNA fragments, antibodies, and other biomolecules comprises the steps of constructing an appropriately labeled beadset, exposing the beadset to a clinical sample, and analyzing the combined sample/beadset by flow cytometry is disclosed. Flow cytometric measurements are used to classify, in real-time, beads within an exposed beadset and textual explanations, based on the accumulated data obtained during real-time analysis, are generated for the user. The inventive technology enables the simultaneous, and automated, detection and interpretation of multiple biomolecules or DNA sequences in real-time while also reducing the cost of performing diagnostic and genetic assays. | | |

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

| | | | | | |
|----|--------------------------|----|--|----|--------------------------|
| AM | Armenia | GB | United Kingdom | MW | Malawi |
| AT | Austria | GE | Georgia | MX | Mexico |
| AU | Australia | GN | Guinea | NE | Niger |
| BB | Barbados | GR | Greece | NL | Netherlands |
| BE | Belgium | HU | Hungary | NO | Norway |
| BF | Burkina Faso | IE | Ireland | NZ | New Zealand |
| BG | Bulgaria | IT | Italy | PL | Poland |
| BJ | Benin | JP | Japan | PT | Portugal |
| BR | Brazil | KE | Kenya | RO | Romania |
| BY | Belarus | KG | Kyrgyzstan | RU | Russian Federation |
| CA | Canada | KP | Democratic People's Republic of Korea | SD | Sudan |
| CF | Central African Republic | KR | Republic of Korea | SE | Sweden |
| CG | Congo | KZ | Kazakhstan | SG | Singapore |
| CH | Switzerland | LI | Liechtenstein | SI | Slovenia |
| CI | Côte d'Ivoire | LK | Sri Lanka | SK | Slovakia |
| CM | Cameroon | LR | Liberia | SN | Senegal |
| CN | China | LT | Lithuania | SZ | Swaziland |
| CS | Czechoslovakia | LU | Luxembourg | TD | Chad |
| CZ | Czech Republic | LV | Latvia | TG | Togo |
| DE | Germany | MC | Monaco | TJ | Tajikistan |
| DK | Denmark | MD | Republic of Moldova | TT | Trinidad and Tobago |
| EE | Estonia | MG | Madagascar | UA | Ukraine |
| ES | Spain | ML | Mali | UG | Uganda |
| FI | Finland | MN | Mongolia | US | United States of America |
| FR | France | MR | Mauritania | UZ | Uzbekistan |
| GA | Gabon | | | VN | Viet Nam |

INTERNATIONAL SEARCH REPORT

International Application No.

PCT/US 96/16198

A. CLASSIFICATION F SUBJECT MATTER
IPC 6 G01N15/10 G01N33/50 G01N15/14 C12Q1/68

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 G01N C12Q

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category * | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|------------|--|-----------------------|
| X | WO 93 02360 A (TRANSMED BIOTECH INCORPORATED) 4 February 1993 | 1-49,53 |
| A | see page 4, line 18-31 see page 5, line 6-10 see page 6, line 10-25 see page 6, line 26 - page 7, line 8 see page 7, line 18 - page 8, line 2 see page 9, line 31-32 see page 19, line 36 - page 20, line 2 --- | 50 |
| A | WO 89 11101 A (MICHAEL JOHN HOLMES) 16 November 1989 see page 5, line 20 - page 6, line 15 see page 7, line 33 - page 8, line 2 see page 8, line 15-20 --- | 50-52 |
| | -/-- | |

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

* Special categories of cited documents :

- *A* document defining the general state of the art which is not considered to be of particular relevance
- *E* earlier document but published on or after the international filing date
- *L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- *O* document referring to an oral disclosure, use, exhibition or other means
- *P* document published prior to the international filing date but later than the priority date claimed

T later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

X document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

Y document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

A document member of the same patent family

Date of the actual completion of the international search

28 July 1997

Date of mailing of the international search report

- 6. 08. 97

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+ 31-70) 340-2040, Tx. 31 651 epo nl,
Fax (+ 31-70) 340-3016

Authorized officer

Zinngrebe, U

INTERNATIONAL SEARCH REPORT

Internatio. Application No
PCT/US 96/16198

| C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT | | |
|--|---|---|
| Category * | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
| A | US 4 499 052 A (FULWYLER) 12 February 1985 see column 1, line 61 - column 2, line 24 see column 6, line 4-11; examples 1,4 --- | 1-5,30, 35,38, 40,42, 46,50-53 |
| A | US 5 028 545 A (SOINI) 2 July 1991 see column 1, line 63 - column 2, line 4 see column 2, line 20-37 see column 2, line 62-66 see column 3, line 10-13 see column 3, line 44-49 --- | 1-5,30, 35,38, 40,42, 46,50-53 |
| A | US 5 380 663 A (SCHWARTZ ET AL) 10 January 1995 see column 1, line 30-56 see column 2, line 18-42 see column 2, line 65 - column 3, line 8 see column 4, line 21-28 see column 5, line 8-30 see column 7, line 30 - column 9 --- | 1-5,30, 35,38, 40,42, 46,50-53 |
| A | US 5 290 707 A (S. WOOD) 1 March 1994 see figure 4 --- | 3-5 |
| A | METHODS IN CELL BIOLOGY, vol. 33, 1 January 1990, pages 613-619, XP000568939 FULWYLER M J ET AL: "FLOW MICROSPHERE IMMUNOASSAY FOR THE QUANTITATIVE AND SIMULTANEOUS DETECTION OF MULTIPLE SOLUBLE ANALYTES" see figure 1E --- | 3-5 |
| A | EP 0 633 462 A (ORTHO DIAGNOSTIC SYSTEMS) 11 January 1995 see page 2, column 42-49 see page 3, line 39-52 see page 5, line 7-9 see page 5, column 57 - page 6, column 15 see page 9, line 4-10 --- | 59 |
| X | US 4 075 462 A (ROWE WILLIAM GUY) 21 February 1978 see column 10, line 11-42; figure 5 --- | 63,64,69 |
| | -/-- | |

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 96/16198

| C(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT | | |
|---|---|-----------------------|
| Category * | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
| A | US 4 987 539 A (MOORE WAYNE A ET AL) 22 January 1991 see column 1, line 1-20 see column 3, line 51 - column 4, line 32 --- | 71 |
| A | WO 92 17853 A (PATTERN RECOGNITION L P) 15 October 1992 see abstract see page 3, line 3-19 see page 5, line 3-19 see page 8, line 13 - page 10 --- | 71 |
| A | US 5 281 517 A (BACUS ET AL.) 25 January 1994 see column 7, line 1-12 see column 8, line 37-52 see column 13, line 27 - column 14, line 3 see column 21, line 62 - column 22, line 15 --- | 72 |
| A | WO 87 06621 A (D. GILLSPIE) 5 November 1987 see page 1, paragraph 2 see page 10, last paragraph - page 11, paragraph 2 see page 12, paragraph 2-5 see page 70, last paragraph - page 73, paragraph 3; figure 6 see page 89, last paragraph - page 90, paragraph 1 --- | 75-77,82 |
| A | EP 0 382 433 A (ICI PLC) 16 August 1990 see page 3, line 3-16 see page 4, line 15-33 see page 5, line 32-57 --- | 75-77,82 |
| A | EP 0 200 113 A (PANDEX LAB INC) 5 November 1986 see page 11, line 19 - page 14, line 26 --- | 75-77,82 |
| A | US 5 104 791 A (ABBOTT SCOT D ET AL) 14 April 1992 see column 1, line 5-10 see column 4, line 20 - column 5, line 18 see column 5, line 34-50 see column 8, line 26-47 see column 9, line 40-46 see column 10, line 3-27 see column 14, line 20-24 ----- | 75-77,82 |

INTERNATIONAL SEARCH REPORT

Intern. application No.

PCT/US 96/ 16198

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. CLAIMS: 1-58,85,89
3. CLAIMS: 71,74

2. CLAIMS: 59-70,71,74
4. CLAIMS: 75-84

FOR FURTHER INFORMATION PLEASE SEE FORM PCT/ISA/206 MAILED 01.04.97

1. ☒ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☒ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

Internatio Application No

PCT/US 96/16198

| Patent document cited in search report | Publication date | Patent family member(s) | Publication date |
|---|---------------------|--|--|
| WO 9302360 A | 04-02-93 | AU 2348092 A CA 2113350 A EP 0594763 A JP 6509417 T US 5567627 A | 23-02-93 04-02-93 04-05-94 20-10-94 22-10-96 |
| WO 8911101 A | 16-11-89 | AT 127231 T AU 3565889 A CA 1335238 A DE 68924064 D DE 68924064 T EP 0413741 A JP 3504276 T US 5585241 A | 15-09-95 29-11-89 18-04-95 05-10-95 08-02-96 27-02-91 19-09-91 17-12-96 |
| US 4499052 A | 12-02-85 | DE 3331017 A FR 2532431 A GB 2126341 A,B JP 1772176 C JP 4047265 B JP 59060261 A US 4717655 A | 08-03-84 02-03-84 21-03-84 14-07-93 03-08-92 06-04-84 05-01-88 |
| US 5028545 A | 02-07-91 | SE 458968 B DE 3870324 A EP 0296136 A JP 1035268 A SE 8702511 A | 22-05-89 27-05-92 21-12-88 06-02-89 17-12-88 |
| US 5380663 A | 10-01-95 | US 5089416 A US 5084394 A US 5093234 A US 4857451 A US 4774189 A US 4767206 A US 5314824 A WO 9100509 A US 5073497 A US 5073498 A US 4918004 A | 18-02-92 28-01-92 03-03-92 15-08-89 27-09-88 30-08-88 24-05-94 10-01-91 17-12-91 17-12-91 17-04-90 |

INTERNATIONAL SEARCH REPORT

Information on patent family members

Internatio. application No

PCT/US 96/16198

| Patent document cited in search report | Publication date | Patent family member(s) | Publication date |
|---|---------------------|--|--|
| US 5380663 A | | GB 2172104 A,B US 4714682 A US 4868126 A JP 6084969 B JP 61228349 A | 10-09-86 22-12-87 19-09-89 26-10-94 11-10-86 |
| US 5290707 A | 01-03-94 | NONE | |
| EP 633462 A | 11-01-95 | CA 2125228 A CA 2141664 A EP 0654160 A WO 9429800 A | 09-12-94 22-12-94 24-05-95 22-12-94 |
| US 4075462 A | 21-02-78 | CA 1050660 A DE 2558392 A FR 2297413 A GB 1497698 A JP 51095884 A | 13-03-79 15-07-76 06-08-76 12-01-78 23-08-76 |
| US 4987539 A | 22-01-91 | AU 2527188 A WO 8900894 A | 01-03-89 09-02-89 |
| WO 9217853 A | 15-10-92 | AU 1791192 A | 02-11-92 |
| US 5281517 A | 25-01-94 | US 5016283 A US 4741043 A US 5018209 A US 5541064 A CA 1312276 A DE 3854644 D DE 3854644 T EP 0317139 A JP 1165958 A US 5086476 A US 4998284 A US 5134662 A DE 3688330 A DE 3689856 D DE 3689856 T EP 0245466 A | 14-05-91 26-04-88 21-05-91 30-07-96 05-01-93 07-12-95 13-06-96 24-05-89 29-06-89 04-02-92 05-03-91 28-07-92 27-05-93 30-06-94 08-09-94 19-11-87 |

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/US 96/16198

| Patent document cited in search report | Publication date | Patent family member(s) | Publication date |
|---|---------------------|----------------------------|---------------------|
| US 5281517 A | | EP 0248840 A | 16-12-87 |
| | | EP 0571053 A | 24-11-93 |
| | | JP 2527536 B | 28-08-96 |
| | | JP 7168102 A | 04-07-95 |
| | | JP 7146289 A | 06-06-95 |
| | | JP 7181179 A | 21-07-95 |
| | | JP 7111425 B | 29-11-95 |
| | | JP 63501597 T | 16-06-88 |
| | | US 4887892 A | 19-12-89 |
| | | WO 8702802 A | 07-05-87 |
| | | WO 8702803 A | 07-05-87 |
| | | US 5109429 A | 28-04-92 |
| | | US 5008185 A | 16-04-91 |
| | | US 5485527 A | 16-01-96 |
| | | US 5272759 A | 21-12-93 |
| | | CA 1326601 A | 01-02-94 |
| WO 8706621 A | 05-11-87 | AT 114334 T | 15-12-94 |
| | | AU 613870 B | 15-08-91 |
| | | AU 7432987 A | 24-11-87 |
| | | CA 1301606 A | 26-05-92 |
| | | DE 3750774 D | 05-01-95 |
| | | DE 3750774 T | 27-04-95 |
| | | EP 0305399 A | 08-03-89 |
| | | JP 2552691 B | 13-11-96 |
| | | JP 1502317 T | 17-08-89 |
| | | US 5482834 A | 09-01-96 |
| EP 0382433 A | 16-08-90 | AU 4902590 A | 16-08-90 |
| | | CA 2009454 A | 07-08-90 |
| | | GB 2228998 A,B | 12-09-90 |
| | | JP 2295496 A | 06-12-90 |
| EP 0200113 A | 05-11-86 | JP 61264240 A | 22-11-86 |
| US 5104791 A | 14-04-92 | NONE | |